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Micelles of Amphiphilic Diblock and Triblock Copolymers Based on Poly(ethylene oxide) and Poly(ε-caprolactone) and Their Potential as Nanocontainers for Drugs

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Two series of MOPEO-*b*-PCL diblock copolymers (DBCs) contained methoxypoly(ethylene oxide) (M_n = 2.5 and 4.54 kDa) and poly(ε-caprolactone) of a variable M_n and also the triblock copolymer (TBC) PCL-*b*-PEO-*b*-PCL based on poly(ethylene oxide) (M_v = 6 kDa) were synthesized and characterized by NMR spectroscopy. Their micellization in water/dioxane solutions was studied using static and dynamic light scattering, UV-Vis spectroscopy and TEM. The micelles of spherical and ellipsoidal morphology, which size and stability in a solution (estimated by *CMC* and $-\Delta G^\circ$ values) grew with increase in the length of both the "core"-forming (PCL) and "corona"-forming (PEO) blocks, were found in the dilute DBC solutions. Unlike this, the "flower-like" micelles of a smaller size and stability occured in TBC solutions. The prospects for application of DBC and TBC micelles as biocompatible and biodegradable carriers for poorly soluble and anticancer drugs (prednisolon, doxorubicin etc.) were considered.

Keywords: Diblock Copolymer, Micellization, Morphology, Nanocarrier, Drug.

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1. INTRODUCTION

In recent years great scientific interest was paid to creation of nanoscaled particulate systems due to their availability as drug carriers for therapeutic applications. Core-shell type micelles of amphiphilic block copolymers with immiscible biocompatible and biodegradable components (e.g. polyethers and polyesters) are ones of the most suitable for this purpose [1, 2]. Polymeric nanoparticles of this kind are able to solubilize various hydrophobic and/or toxic drugs, minimize drug degradation and loss upon administration, prevent harmful side effects and provide safe target transport in the living organisms [3, 4]. The final efficiency of such therapeutic systems mostly depends on the carrier parameters: morphology, critical micelle concentration, particle size distribution and micelle stability, as well as on the way of drug incorporation and the method of preparation of the delivery system [5]. The present work is dedicated to the studying of micellar nanocontainers, obtained by the self-assembling of block copolymers containing methoxypoly (ethylene oxide) (MOPEO) or poly(ethylene oxide) (PEO) and poly(ε-caprolactone) (PCL), which are well known for their nontoxic nature and biocompatibility. Our investigations were directed to cover the above-mentioned aspects of micellization processes in these block copolymer solutions.

2. EXPERIMENTAL

2.1 Materials and Syntheses

The ε-caprolactone (CL) from "Sigma-Aldrich" (USA), two samples of methoxypoly(ethylene glycol) (MOPEG) with M_v = 2 kDa and 5 kDa from "Fluka" (USA) and poly(ethylene glycol) (PEG) with M_n = 6 kDa from "Aldrich" (USA) were used to synthesize two series of diblock copolymers MOPEO-*b*-PCL (DBCs) and triblock copolymer PCL-*b*-PEO-*b*-PCL (TBC) correspondingly by the method of anionic ring-opening block copolymerization with stannous octoate ($Sn(Oct)_2$) from "Acros Organics" (USA) as initiator. The methodology of DBC synthesis was reported earlier [6]. In the case of TBC synthesis the molar ratio was $[Sn(Oct)_2]/[PEG] = 2$ (unlike the DBCs synthesis, where $[Sn(Oct)_2]/[MOPEG] = 1$.) in order to initiate both ends of PEG chain. To define a chemical structure and molecular parameters of synthesized block copolymers the ^1H NMR spectra of all DBCs, TBC and initial MOPEGs, recorded in CCl_4 at 20 °C by a 400 MHz "Mercury-400" spectrometer from "Varian" (USA), was used.

2.2 Micelle Characterization

The micelle formation processes of DBC and TBC macromolecules in dioxane/aqueous solutions were studied using UV-Vis spectroscopy. The optical density (turbidity) of all the solutions was measured at a room temperature and λ = 500 nm with UV-Visible Spectrophotometer "Varian Cary 50 Scan". The determination of the critical micellization concentrations (*CMC*s) in copolymer micellar solutions by static light scattering (SLS) was performed on a modernized instrument "FPS-3" (Russia), equipped with a light-emitting diode WP7113VGC/A from "Kingbright", the controller ADC-CPU™ from "Insoftus" (Ukraine) and the computer program "WINRECORDER". The scattering intensities (I_v) of the vertically polarized incident light with λ = 520 nm were obtained at the scattering angle θ = 90° in a vide region of the copolymer concentrations.

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TEM images of the copolymer micelles were recorded with a JEM-I23O instrument ("JEOL", Japan) operating at an accelerating voltage of 90 kV. Small drops (~$1{\cdot}10^{-4}$ cm^3) of the copolymer solutions (C_{DBC} = 0.3, C_{TBC} = 0.3 kg·m^{-3}) in the deionized water were deposited in copper grids coated with Formvar film and carbon and then were dried for ~1 min at 50 °C.

Dimensions of DBC and TBC micelles as a function of the length of MOPEO and PCL blocks were determined by dynamic light scattering (DLS) using a ZetaSizer 3 instrument ("Malvern", UK). A He-Ne laser operating at λ = 632.8 nm was used as a light source. The autocorrelation function of scattered light was measured in aqueous micellar solutions at the copolymer concentration higher than *CMC* (for DBC2, DBC3 C = 0.1 kg·m^{-3}; for DBC4, DBC5 C = 0.24 kg·m^{-3} and 0.3 kg·m^{-3} for DBC6, TBC), the θ = 90° scattering angle and T = 20 °C. For this purpose, a preliminary dialysis of the copolymer dioxane/aqueous (30/70 v/v) solutions against water was performed for two weeks. In every solution 10-12 parallel measurements were carried out. The results of DLS were analyzed by CONTIN algorithm (PCS program: Size mode v.1.61).

3. RESULTS AND DISCUSSION

3.1 Molecular Parameters of Block Copolymers

Chemical structure of DBCs and TBC and molecular weights of PCL blocks and whole macromolecules were characterized by the ^{1}H NMR spectroscopy. The interpretation example of NMR spectra of obtained block copolymers was discussed earlier [6]. Macromolecular parameters for two series of MOPEO-*b*-PCL and for PCL-*b*-PEO-*b*-PCL (Table 1) were calculated using the data from their ^{1}H NMR spectra according to the following equations – (1),(2) for DBCs and (3) for TBC:

$$M_{nMOPEO} = \frac{3 \cdot M_{0MOPEG} \cdot A_a}{4 \cdot A_b} \quad (1)$$

$$M_{nPCL} = \frac{2 \cdot M_{0PCL} \cdot M_{nMOPEO} \cdot A_c}{M_{0MOPEG} \cdot A_a} \quad (2)$$

$$M_{nPCL} = \frac{M_{0PCL} \cdot M_{nPEO} \cdot A_c}{M_{0PEG} \cdot A_a} \quad (3)$$

where M_{nMOPEO} (M_{nPEO} for TBC) and M_{nPCL} are the number average molecular weights of MOPEO (PEO) and PCL blocks, M_{oMOPEG} (M_{oPEG} for TBC), and M_{oPCL} are the molecular weights of MOPEG (PEG) and PCL units, A_a, A_b are the integral intensities of the signals of protons of methylene and terminal methoxy groups correspondingly in MOPEO (PEO) blocks, and A_c is the integral intensity of proton signal of methylene group near carbonyl in the PCL unit.

It is seen from Table 1, that the length of hydrophilic MOPEO block remains constant for each of two DBC series while the hydrophobic PCL block length varies.

Table 1 – Macromolecular parameters of DBCs and TBC

Sample	M_{n}MOPEO/PEO, kDa	M_{n}PCL, kDa	M_{n}DBC/TBC[a)], kDa	n[b)]
DBC1	2.5	2.8	5.3	0.42
DBC2	2.5	8.0	10.5	1.25
DBC3	2.5	23.9	24.6	3.68
DBC4	4.5	14.5	19.0	1.24
DBC5	4.5	17.4	21.9	1.48
DBC6	4.5	19.0	23.5	1.62
TBC	6.0	4.9	15.8	0.32

a) M_{n}DBC = M_{n}MOPEO + M_{n}PCL; M_{n}TBC = M_{n}PEO + 2 · M_{n}PCL.
b) The ratio between units of PCL and MOPEO/PEO blocks.

Moreover, the TBC copolymer contains the longest hydrophilic PEO block, which is the central block of the macromolecular chain, and two short side hydrophobic PCL blocks.

3.2 Micellization Process in Mixed Solvent

Taking into account, that dioxane was a good solvent for whole block copolymer chains and water was able to dissolve selectively MOPEO and PEO blocks, we initiated the self-assembly of DBC and TBC macromolecules by a sequence addition of water in dioxane solutions of the copolymers.The optical density (turbidity) for the DBCs and TBC samples in dioxane/aqueous solutions with constant copolymer concentration ($C_{DBC/TBC}$ = 0.8 kg·m^{-3}) was measured in the visible region of the spectrum at a different mixed solvent composition (Fig. 1a,b). The increase of the turbidity corresponds to the development of macromolecular self-assembly in micellar structures with increasing in the volume fraction of water in the block copolymer solutions.

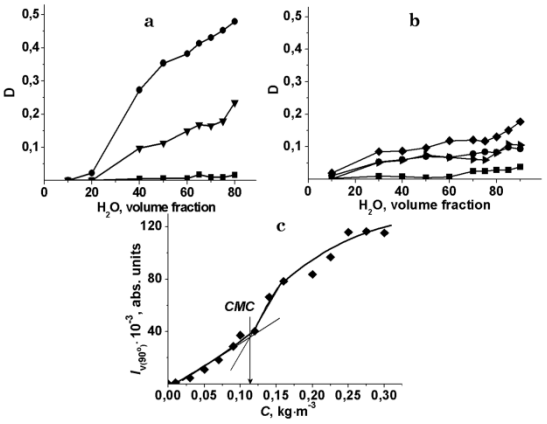

Fig. 1 – Dependences of the turbidity of copolymer ■ = DBC1, ▼ = DBC2, ● = DBC3 solutions (**a**) and copolymer ► = DBC4, ● = DBC5, ♦ = DBC6 and ■ = TBC solutions (**b**) *vs* water content in the mixed dioxane/aqueous solvent; the example of *CMC* determination for DBC6 in dioxane/H_2O (30/70v/v) solution by SLS (**c**)

Furthermore, the tendency to significant intensification of the micellization process with the lengthening of hydrophobic PCL blocks was observed. Indeed, the optical density in case of TBC and DBC1 copolymers with shortest PCL blocks was noticeably lower then for other samples. It is necessary to note that the turbidity intension of DBC4, DBC5 and DBC6 solutions (Fig. 1b)

was lower in comparison with DBC2 and DBC3 (Fig. 1a), which could be attributed to the bigger length of water-soluble MOPEO block in DBC4-DBC6 series. In general, the micelle formation process occurred in typical way for amphiphilic copolymers in selective solvents, namely hydrophobic micellar "core" was formed by PCL blocks whilst MOPEO or PEO (for TBC) blocks created the stabilizing "corona". The PCL "core" is able to be a medium for water-insoluble drugs bound by copolymer micelles [7]. FTIR spectrum of DBC micelles blend with model hydrophobic drug prednisolon (PS) (the data are not shown), unlike the spectra of pure DBC and PS, demonstrated two new intense $\nu_{O\text{-}H}$ vibration bands at 3284 and 3358 cm^{-1}, which confirms the hydrogen bonds existence between DBC ether or/and ester groups and PS hydroxyls. Thus, DBC micelles incorporate drug by means of H-bonding and additional hydrophobic interactions.

For the further determination of the termodinamic parameters of DBC and TBC micellization a constant composition of the mixed solvent (dioxane/H_2O = 30/70 v/v %) was chosen. The critical micelle concentration (*CMC*) and the Gibbs free micellization energy ($-\Delta G°$) are the important parameters for characterization of micellar system because they reflect the size of micelles and their stability, which are significant for biomedical application of block copolymers as drug carriers [5, 7]. The static light scattering was applied to establish the *CMC* values for DBC2–DBC6 and TBC (Table 2) as it is shown in Fig. 1c. Using thermodynamic theory for micelle formation [7] the $-\Delta G°$ values were calculated by the functional dependence $\Delta G° = RT \cdot \ln CMC$ (Table 2).

Table 2 – Thermodynamic parameters of micelle formation

Copolymer	$CMC \cdot 10^5$, mol · dm^{-3}	$-\Delta G°$, kJ · mol^{-1}
DBC2	0.54	29.75
DBC3	0.07	34.85
DBC4	0.92	28.44
DBC5	0.64	29.33
DBC6	0.46	30.14
TBC	1.9	26.66

The data in Table 2, demonstrate the regular reduction in *CMC* values and the respective increase in $-\Delta G°$ with growth of the hydrophobic PCL block length for each of DBC series. Thus, the stability of the diblock copolymer micellar structures in the selective solvent increases with lengthening of "core"-forming PCL block. The highest *CMC* and the lowest $-\Delta G°$ for TBC (PCL-*b*-PEO-*b*-PCL) indicate the smallest micelle stability in comparison with all DBC samples.

3.3 Micelle Size and Morphology

For all copolymer samples excluding DBC2, the autocorrelation function of scattered light, measured in their aqueous solutions by DLS was a single-exponential type decay curve. In this case, the decay rates (Γ) of this function formed monomodal distribution and were connected with corresponding translation diffusion coefficients (D) of micelles by the relation [8, 9]: $\Gamma = D \cdot q^2$, where q is the scattering vector. From the diffusion coefficient distribution, the distribution on the hydrodynamic radius (R_h) or diameter (d_h) of the micelles with spherical shape could be calculated using the Stokes-Einstein equation [8,9]: $D = k_B T / 6\pi \cdot \eta \cdot R_h$, where k_B is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity. The example of monomodal distributions on d_h, which were determined in some parallel measurements for one of the copolymer samples, is shown in Figure 2.

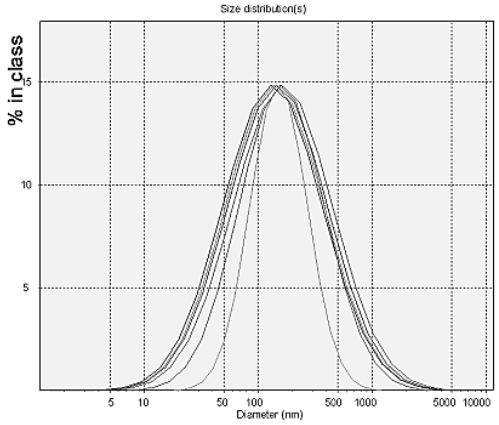

Fig. 2 – The monomodal size distributions on the micelle diameter for DBC3 aqueous solution, $C = 0.1$ kg · m^{-3}.

In DBC2 solution, where the copolymer concentration was low, the correlation function was the Multi-exponential type decay curve. Thus, it was shown that this solution contained side by side with copolymer micelles also individual DBC2 molecules, which size was found to be 2 nm. The results of DLS studies are collected in Table 3.

Table 3 – The average dimensions of block copolymer micelles

Copolymer	d_{av} [a], nm	$d_{av(i)}$ [b], nm	$d_{av(v)}$ [c], nm	$d_{av(n)}$ [d], nm
DBC2	141 ± 26	167	123	82
DBC3	150 ± 12	182	140	89
DBC4	104 ± 38	141	47	27
DBC5	113 ± 22	150	49	27
DBC6	163 ± 10	175	160	138
TBC	66 ± 21	95	77	64

The average hydrodynamic diameters of micelles calculated on [a]monomodal distribution, [b]scattered intensity, [c]micelle volume and [d]micelle number.

It should be noted, that the samples DBC2-DBC3 and DBC4-DBC6 constitute two copolymer series with constant (but different) length of MOPEO blocks and increasing length of PCL blocks (Table 1). According to Table 3, in both these series the average hydrodynamic diameter of micelles regularly growth with increase in the length of hydrophobic "core"-forming PCL blocks, which correlates with the data of other studies [7, 10]. Also, it is agreed with the changes in the micelle stability (in *CMC* and $-\Delta G°$ values) in both the copolymer series, which were discussed above (Table 2). The influence of hydrophilic "corona"-forming MOPEO block length on micelle dimensions could be established by comparison of the data in Table 3 for DBC3 and DBC6 samples, which contain commensurable PCL blocks and differ mainly by growing length of MOPEO blocks. It is clear, that the size of micelles (Table 3)

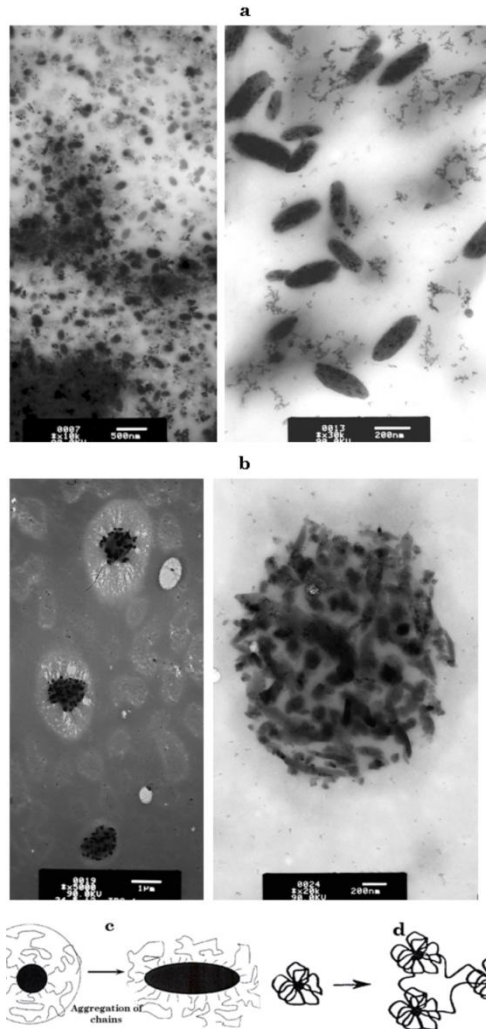

Fig. 2 – TEM images with a lesser (left) and higher (right) enlargement for micellar structures of DBC6 (a) and TBC (b); the schematic representation of spherical and ellipsoid micelles of DBC6 (c) and one "flower-like" micelle of TBC and its micellar aggregate with some hydrophobic centers (d).

essentially grow at the lengthening of hydrophilic blocks. The smallest size and stability in a solution are characteristic for the micelles of TBC. This copolymer sample includes not only two shortest hydrophobic blocks and the longest hydrophilic block but also possesses so-called "telechelic" molecular building, when one hydrophilic-block places in the center of macromolecule and two hydrophobic blocks (or volume groups) are on each side. In this case, the formation of special "flower-like" micelles is possible [11, 12].

Morphology of DBC and TBC micelles together with their schematic representations are shown in Figure 3. These results are of significant interest. Indeed, the studied DBC sample demonstrates as traditional spherical morphology of its micelles as unusual ellipsoidal shape (Figure 3a,c). The appearance of similar elongated micellar structures was also observed in the study [13] for other amphiphilic diblock copolymer and was interpreted by strong polydispersity of hydrophilic "corona"-forming blocks. We did not estimate the polydisperisty index for initial commercial samples of MOPEG, which were used in DBC syntheses. Therefore, the role of this factor in the formation of ellipsoidal DBC micelles is not clear yet. But in any case, such elongated micelles could be considered as intermediate structures between spherical and "rod-like" or "worm-like" micelles.

Interesting micellar structures are displayed in TEM images for TBC (Figure 3b). Their size is essentially higher than that found for the same TBC sample by dynamic light scattering just after dialysis (Table 3). Moreover, their spherical construction is unusual (as compared to DBC micelles) because contains some hydrophobic "cores". At the same time, the appearance of exactly such micellar structures as a result of aggregation of "flower-like" micelles was predicted in some studies devoted to the "telechelic" polymers [11,12]. Being based on these works and taking into account a large time interval (about 25 days) between our light scattering and TEM experiments, we also explaine the rise of large spherical TBC micelles, comprised by numerous hgydrophobic "cores", by the aggregation of small initial "flower-like" micellar structures (Figure 3d).

CONCLUSION

The amphiphilic MOPEO-*b*-PCL copolymers with biocompatible and biodegradable blocks in selective solvent form micelles of spherical and uncommon ellipsoidal morphology, which size and stability increase with the lengthening of "core"-forming hydrophobic PCL block. The length of hydrophilic MOPEO block also affects micellar structure morphology, namely longer MOPEO bloks upsize DBC micelles. The triblock copolymer PCL-*b*-PEO-*b*-PCL with "telechelic" molecular building forms "flower-like" micelles of the smallest size and lower stability, but after a time they combine in large spherical micelles with numerous hydrophobic "cores". DBC and TBC micelles possess characteristics that are suitable for their further application as drug nanocarriers.

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